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Q Fever and *Coxiella burnetii*: a Model for Host-Parasite Interactions†

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HISTORY

In 1935 an outbreak of a fever of unknown etiology occurred among abattoir workers in Brisbane, Australia. R. Cilento, Director-General of Health and Medical Services for Queensland, requested E. H. Derrick, Director of the Laboratory of Microbiology and Pathology of the Queensland Health Department at Brisbane, to investigate the nature of the disease. Derrick's classic paper (54) provided the name "Q" (for Query) fever for the disease and accurately described most of the clinical features of the infection. A subsequent review by Derrick (55) collated data from the time of the first description of the disease in 1935.

Q fever displayed certain clinical similarities to typhus fever and to typhoid and paratyphoid fevers, but the absence of a rash and a negative Weil-Felix reaction ruled out these infections. Although Derrick was unable to identify or isolate the etiological agent, he succeeded in transmitting Q fever to guinea pigs with the blood or urine of infected patients (54). As with humans, guinea pigs developed a long-lived immunity to the infection. Derrick sent a sample of infected guinea pig liver to Burnet, who used it to transmit Q fever to guinea pigs and mice. Burnet identified "typical rickettsiae" in infected mouse spleen impression smears (35); the organisms were ". . . less than 1 μ in length and about 0.3 μ across; the shape varied from . . . rods to coccoid forms" (35). In closely reasoned arguments, Burnet concluded that the agent of Q fever was a rickettsial agent unlike the typhus, scrub typhus, or spotted fever rickettsiae. The papers of Derrick and of Burnet and Freeman remain models of careful investigations, critical analyses, and conclusions. In 1938 a series of papers described a "filter-passing infectious agent" from *Dermacentor andersoni*, ticks recovered near Nine Mile Creek, 32 miles west of Missoula, Mont. (48, 52, 61). Guinea pigs infected with the agent developed high fever and enlarged spleens. The organism occurred intravacuolarly in infected cells, and the vacuoliza-

† This review is dedicated to Cora M. Downs, scientist, teacher, and friend.

tion could be so great as to force the cell nucleus toward the edge of the cell. A minute pleomorphic organism was isolated which stained deeply with the Macchiavello and Giemsa techniques (73). A laboratory worker ("case X") became infected and developed the symptoms of Q fever as originally described by Derrick and Burnet. Blood from case X successfully infected a guinea pig, whose spleen was infectious for other guinea pigs. Cox concluded that the agent either was a rickettsia or was rickettsia-like. Dyer (62) proposed the identity between the Q fever agent of Derrick and Burnet and the filter-passing agent, or "X virus," and subsequently Cox (48) proposed the name *Rickettsia diaporica*, incorporating the rickettsial features of the organism with its ability to flow through the pores of a bacteriological filter. These classic papers established the presence of Q fever in the United States and were the background for the subsequent nomenclature of *Coxiella burnetii*.

Although Derrick and Burnet originally described the organism as a rickettsia (*Rickettsia burneti*), it is now clear that *C. burnetii* differs from "typical" rickettsiae, such as *R. prowazeki*, *R. typhi*, *R. akari*, and *R. rickettsii*, on the basis of genome guanine-plus-cytosine content, vector of transmission, nature of intracellular proliferation, size and pleomorphic nature, sensitivity to antibiotics, and greater resistance to higher temperature and lower pH (63, 81, 82). The genome size of *C. burnetii* is 1.04×10^9 daltons, the same magnitude as that of *R. prowazeki*, *R. rickettsii*, and *R. typhi* (138). An earlier estimate of 1.8×10^7 daltons (175) was probably based on a heterogeneity of sheared DNA fragments. The 42.2% guanine-plus-cytosine content of *C. burnetii* DNA is strikingly different from that of typical rickettsiae; the guanine-plus-cytosine compositions of some rickettsial DNAs are as follows: *R. prowazekii* (Breinl) and *R. typhi* (Wilmington), 29.0% (138); *R. canada*, 29.3 to 30.3%; *R. rickettsii*, 32.0 to 33.2%; *R. conori*, 32.9 to 33.3% (196). As described below, *C. burnetii* proliferates in a wide range of vertebrate and invertebrate hosts and infects humans via aerosol inhalation, milk and meat ingestion, contact with infected blood, and arthropod and nonarthropod vectors. The agent proliferates within lysosomal vacuoles in the cytoplasm (14, 38), in contrast to the replication patterns of most typical rickettsiae. Based on chicken embryo infection and protection assays, *C. burnetii* is relatively insensitive to erythromycin, chloramphenicol, and thiocymetin as compared with typical rickettsiae (149). Nevertheless, the Giemsa and Macchiavello staining response and the obligate intracellular parasitic nature of the organism justify its taxonomic position in the *Rickettsiaceae* and, as a separate genus, *Coxiella*. A wealth of epidemiological, clinical, and immunological information on Q fever and on the biology of *C. burnetii* has been gathered. Comprehensive reviews are available (10, 11, 34, 76, 144, 147, 203, 204, 216). The discussions of Brezina (31) and Weiss (203, 204) should also be consulted.

The typical picture of Q fever in humans (35, 54, 55) is an incubation period of 15 days or less, varying with route of exposure, dosage of rickettsiae, and age of victim. A febrile onset reaches a plateau of 40°C within 2 to 4 days, later accompanied by malaise, anorexia, muscular pain, weakness, and intense (usually) preorbital headache. The headache later becomes generalized, continuing in intensity throughout the disease. A gradual defervescence occurs over a 1 to 2 week period, although the fever may last longer in older patients and may display biphasic peaks. Liver damage accompanied by hepatomegaly occurs, with frequent reports of hepatic granulomas (see below). A pneumonitis and bronchitis with a dry unproductive cough are often symptomatic, leading to erroneous diagnoses of influenza or an influenza-like disease (the "Balkan grippe" or "Balkan fever" of World War II and the "Termez fever" in Tashkent). Derrick (54) had noted that, whereas the cases showed no palpable spleens, they were probably "enlarged to some extent in human cases." This prescient comment later proved accurate, and subsequently both hepatomegaly and splenomegaly were found in human cases and were characteristic of experimentally infected guinea pigs (158). In human cases a rickettsemia is present in the early stages of fever, and the rickettsiae may be found in urine at this time. Rickettsiae disappear from the blood with defervescence, but kidneys and heart valves may remain latently infected. Endocarditis, although not common, may be a sequela of infection (see below).

A long-lived immunity generally results from the infection, although chronic and latent infections have been reported (180, 181). Q fever is managed by tetracycline and chloramphenicol therapy (72), but *C. burnetii* is less sensitive to these antibiotics than typical rickettsioses (149). Intravenous administration of erythromycin may be an especially effective treatment (51). Q fever remains endemic in Australia where it has continued to be a major problem among livestock workers (79).

Reported human cases of Q fever steadily increased in the United States between 1948 and 1977 (50). Q fever had become endemic among dairy herds throughout the United States by 1960 (122). The presence of *C. burnetii* in milk and the knowledge that humans could become infected by drinking such milk led to a critical

study of conditions for thermal inactivation of the rickettsia. The pasteurization procedures of the time (143°F [61.7°C], 30 min) were based on the thermal death point of *Mycobacterium tuberculosis* and were inadequate for complete killing of *C. burnetii*. Heating contaminated milk at 161°F (71.7°C) for 15 s killed all rickettsiae, and in 1956 the Assistant Surgeon General of the U.S. Public Health Service wrote to all state and territorial milk control authorities, recommending these conditions for milk pasteurization; such conditions were then generally adopted (63).

More than 4 decades after the first reports of the disease, Q fever has been found to be distributed worldwide and remains a potential hazard in almost any habitable region of the earth.

EPIDEMIOLOGY

Since its first description in Australia, Q fever has been found throughout the world, except for the Antarctic regions (96). The agent has been identified in arthropods, fish, birds, rodents, marsupials, and livestock. In the United States, Q fever has been reported to the Centers for Disease Control in Atlanta from 31 states (50). Although as of 1979 the disease was not on the list of nationally notifiable diseases, 26 states required notification of human cases and 5 others required the reporting of cases in animals (50). In a survey conducted by the Centers for Disease Control of the 1,164 Q fever cases reported between 1948 and 1977, 67% were from California (50). No cases had been reported to the Centers for Disease Control before 1948. It is not surprising that relatively few cases of Q fever have been reported considering that this disease resembles other diseases such as viral influenza, the inability to cultivate the agent in vitro, and the tendency on the part of clinicians and public health officials to regard it as an unimportant disease. The actual number of cases far exceeds those reported. For example, Bell et al. (19) conducted a serological survey in the Los Angeles area and concluded that more than 50,000 persons had been infected with the agent during a 2-year span in the 1940s. During a 2-year span approximately 20,000 cases had occurred in Italy (11).

The principal modes of entry of the agent into humans are via inhalation of contaminated dust particles and aerosols generated in the milieu of abattoirs and dairies and the ingestion and handling of infected meat and milk (20, 22, 53, 129, 187). The agent originates from the major reservoirs of *C. burnetii*—dairy cows, sheep, and goats. Infected cows shed enormous numbers of rickettsiae in their milk, birth fluids, and colostrum although appearing healthy (22, 91, 123, 187). In southern California, with up to 98% of dairy herds seropositive for Q fever, 300 human cases were also reported, and 350 human cases were associated with Q fever reservoirs in sheep in northern California. Similar correlations were found in Pennsylvania (130). In 1974 82% of tested dairy cattle in California were seropositive for *C. burnetii*, and the highest rate occurred in southern California. Rickettsiae were shed in the milk of 51% of a large group of tested cows. The data represented a sevenfold increase over a 25-year period (22). Similar studies on goats in California in 1977 showed 24% seropositive reactions, and 7% of the animals shed the organisms in their milk (173). Q fever has also been contracted by handling infected carcasses or placentas (123, 136, 163, 207) or laboratory material (145, 216). The agent has been found in human placentas (69), presenting a hazard for midwives and in obstetric theatres, especially in underdeveloped countries.

Babudieri (11) reviewed in detail the zoonotic aspects of Q fever. The agent naturally infects over 40 species (including 12 genera) of ticks found in five continents (11). Some additional arthropods have been found infected with *C. burnetii* (11). Other arthropods, including fleas, lice, and cockroaches, have been experimentally infected; their role in the maintenance of Q fever in nature is unknown. The role of ticks in the transmission of the rickettsia is also not known, although they probably transmit the parasites within domestic and wild animals and from wild animals to domestic animals (11). Existence of a kangaroo-tick cycle in western Queensland, Australia, was suggested by the discovery of infected kangaroos and infective kangaroo ticks, *Amblyomma triguttatum*, from kangaroos, goats, and sheep (164). Ticks were further implicated in outbreaks of Q fever among sheep shearers.

Several wild animals have been reported to be infected with *C. burnetii*, including bandicoots in Australia (56, 70) and desert rats, wild rabbits, and mice in North Africa (11). The rickettsia has been isolated from several bird species, including pigeons and sparrows in Europe and Asia, and in some cases from their ectoparasitic ticks and mites (11). Because most of the infected birds reported have close contact with humans and domestic animals, it is thought (84) that they are not involved in the transmission of the disease to humans, but are rather indicators of the agent in humans.

In none of the animals, wild or domestic, does it appear that the Q fever agent causes overt disease. In addition, the role of wild animals and arthropod vectors vis-a-vis disease in humans is probably of minor significance.

Q fever in domestic animals is of major impor-

tance and continues to be the principal source of the infection in humans. Although cattle, sheep, and goats are the main reservoirs, the agent has been detected as well in dogs, camels, buffaloes, and geese and other fowl (11).

Cases of transmission of the agent from human to human have been reported but are quite rare; the isolation of Q fever patients is not called for. In one rare case, Q fever was acquired by personnel participating in the necropsy of a fatal case of Q fever (129).

Recent outbreaks of Q fever at research institutions underscore the importance of domestic animals, specifically sheep, in the spread of the agent. In one outbreak involving 19 confirmed cases and 68 presumptive cases, it was suspected that pregnant ewes being used for prenatal research were the source of infection (40). In a second outbreak involving 81 persons, pregnant sheep also being used in prenatal research were the source of infection (136). Transmission was directly linked to rooms where the sheep were held and the corridors through which they were transported. Affected individuals included personnel working directly with infected placentas and individuals who walked past sheep held in a corridor.

CULTIVATION AND GROWTH OF *C. BURNETII*

Embryonated hens' eggs serve as excellent hosts for cultivating large numbers of the parasite. Typically, 5- to 7-day-old embryos are inoculated via the yolk sac and incubated at 35°C for an additional 10 to 12 days, during which time the rickettsiae proliferate luxuriantly within the yolk sac and sparsely in the rest of the embryo (146). Multiplication of *C. burnetii* was studied after inoculation into yolk sacs of embryonated eggs (121). At 11 days post-inoculation, highest numbers of rickettsiae were found in the yolk sac membrane, and large numbers were also present in the embryo's intestines, chorioallantoic membrane, allantoamnion, and amniotic fluid. *C. burnetii* was present in lower numbers in embryonic muscles, liver, spleen, and heart.

Purification of the rickettsiae from host components is a laborious process involving homogenization of infected yolk sacs and differential centrifugation, followed by passage of the semipurified organisms through density gradients. The essential steps have involved the use of the adsorbent Celite and trypsin treatment of the rickettsial suspensions, followed by passage through sucrose gradients (157, 167, 192). Centrifugation through a Renografin density gradient has also been used as a final step in purification (208, 210a). A 1- to 2-g (wet weight) amount of purified rickettsiae can be obtained from 8 dozen eggs.

Entry into and Proliferation Within Cultured Cells; Comparison with Other Rickettsiae

C. burnetii has been cultivated in a variety of animal cells, both primary and established cell lines. Some of these cells are chicken embryo cells, L cells, mosquito cells, human embryo fibroblasts, green monkey kidney (Vero) cells, tick tissue cultures, and the J774 and P388D1 macrophage-like tumor cell lines (14, 38, 166, 171, 206, 215).

Within several of the cell lines, *C. burnetii* establishes a persistent infection, i.e., dividing infected populations that have been maintained for periods exceeding 1 year without the addition of normal cells (14, 38, 168, 215; J. A. Stueckemann, Ph.D. thesis, University of Kansas, Lawrence, 1976).

Entry of the parasite into the cells is almost certainly a passive event on the part of the parasite and occurs by phagocytosis (36). This is unlike other rickettsial species that actively promote their entry into the host cell. The classic work of Cohn et al. (44) showed that metabolic inhibitors caused a marked reduction in the penetration of mouse cells by *Rickettsia tsutsugamushi*. Inactivation of *R. tsutsugamushi* by heat, UV irradiation, and Formalin also prevented cell penetration. Inactivation of *C. burnetii* by heat or Formalin, however, did not affect its uptake by mouse L-929 cells (O. G. Baca, personal communication). Internalization of *R. prowazeki* into L cells required the active participation of both the rickettsia and the host cell (200). It has been proposed that internalization of *R. prowazeki* occurs through a process of "induced phagocytosis": attachment of live rickettsiae to an unidentified site signals the cell to phagocytize (200). Inactive typhus rickettsiae adhered to the cell membrane but internalized slowly.

Studies on the nature of the *C. burnetii* attachment site on the L-929 cell surface showed that only pronase and subtilisin treatment of the L cells significantly inhibited attachment of *C. burnetii* (Baca, personal communication). This provided circumstantial evidence that proteins are at or near the site of *Coxiella* attachment. Whether or not there is a specific component to which the parasite attaches is as yet unknown. It was also found that phase II *C. burnetii* (see "Phase Variation" in "Biology of *C. burnetii*," below) attached to the L cell much more readily than phase I rickettsiae, probably accounting in part for the ability of phase II rickettsiae to more quickly infect various cells, including the L-929 cell. Because lipopolysaccharide (LPS) is a virulence factor of gram-negative bacteria, it is likely

that the LPS, which occurs in greater amounts in phase I than in phase II *C. burnetii*, plays a role in hindering the entry of phase I *Coxiella*. Indeed, adding purified phase I LPS to *C. burnetii* (either phase) and L cells caused a slight reduction in rickettsial attachment and markedly reduced their entry. Whether or not the reduced entry was due to toxicity of the LPS is not known.

There are basic differences between *C. burnetii* and other rickettsial species with respect to localization within host cells. All members of the genus *Rickettsia* grow within the cytoplasm of cells with no apparent association with vacuoles or closely opposed host-derived membranes (5, 183, 205). *C. burnetii*, however, proliferates within vacuoles (23, 36, 206). Electron microscopic studies of infected guinea pig liver revealed numerous vacuoles containing the rickettsiae (83). Occasionally, *C. burnetii* has been observed "free" in the cytoplasm (36, 83). The possibility that these free rickettsiae may have a closely opposed host membrane has not been excluded. *R. rickettsii* and *R. tsutsugamushi* multiply primarily in the cytoplasm, free of host membranes (29, 174). These differences in localization may reflect different modes of entry. The rickettsiae mentioned, other than *C. burnetii*, may be entering by (i) direct penetration into the cytoplasm or (ii) via phagocytosis with subsequent release into the cytoplasm or (iii) by a combination of the two. Immediately after interiorization within vacuoles, they may lyse the phagosomal membrane and escape into the cytoplasm (for a review on the latter, see 212). Once entry into the cell is achieved, both phases of *C. burnetii* proliferate within vacuoles which eventually fuse and form a single vacuole which occupies most of the cell's volume (Fig. 1). The infected cell attains a size several diameters greater than uninfected cells (14, 38); the nucleus and cytoplasm become displaced to the cell's periphery (38, 206; Stueckemann, Ph.D. thesis). When white mice were intranasally and intraperitoneally infected, the rickettsiae grew within vacuoles of the lung alveolar macrophages and of spleen reticular cells (8), resembling the rickettsial growth in guinea pig liver (83) and in L cells (36, 38). Copelovici et al. (47) found similar intravacuolar growth in fibroblasts of human embryonic cells and, on the basis of histochemical reactions, showed that RNA content of nucleoli of infected cells increased. Ariel et al. (8) proposed that the vacuoles themselves, not the rickettsiae, contributed to the pathogenicity and also supported the phagolysomal origin of the vacuoles, proposing that vacuole formation was a cellular defensive response to infection.

Cytochemical investigations revealed that the rickettsiae-containing vacuoles are phagolysosomes (36, 38). Lysosomes labeled with electron-dense thorium dioxide eventually fused with rickettsia-containing phagosomes, and acid phosphatase, a lysosomal enzyme, was detected in the vacuoles. Recently evidence was obtained, using fluorescing lysosomotropic dyes, that the pH of the rickettsiae-containing vacuoles is ca. 5.1, indicative of the presence of the acidic contents of the lysosome (E. Akporiaye and O. G. Baca, unpublished data).

Enumeration

Predictably for a small obligate intracellular parasite, the enumeration of viable or total rickettsiae has presented technical problems. A number of methods have been developed, including the estimation of 50% endpoints by using embryonated eggs, direct microscopic counts of acridine orange-stained rickettsiae mixed with known amounts of marker bacteria (182, 184), and plaque assay (133, 151, 209). All of these procedures are tedious and time-consuming; those using embryos and cell cultures are difficult to reproduce. The plaque assay technique, which utilizes primary chicken embryo cells for estimating viable *C. burnetii*, results in detectable plaques only after 16 days of incubation.

BIOLOGY OF *C. BURNETII*

Ultrastructure/Morphology

The etiological agent of Q fever is highly pleomorphic, coccobacillary in shape with approximate dimensions of 0.3 by 1.0 μm (35), bounded by an envelope similar to those found in gram-negative bacteria (37, 143). The envelope consists of an outer and inner membrane, each approximately 6.5 nm thick. Sandwiched between the membranes and associated with the inner surface of the outer membrane is an electron-dense layer which, although it has the approximate dimensions and location of a typical gram-negative peptidoglycan layer, is insensitive to lysozyme-EDTA treatment, as assessed by electron microscopy (37). Purified "cell walls" from *C. burnetii*, however, dissolve when exposed to lysozyme (162). That peptidoglycan is a part of the *C. burnetii* envelope is strongly suggested by several observations, including: solubilization of purified cell walls in the presence of lysozyme and EDTA (162); the demonstration of muramic acid (3) and diaminopimelic acid (140) in whole cells, key components of typical peptidoglycan.

Depending on the modification of the Gram stain used, *C. burnetii* stains gram negative, gram positive, or gram variable (74). Use of ethyl alcohol-iodine as the mordant results in a strong gram-positive reaction; other rickettsial species stain gram negative. Nevertheless, ultra-

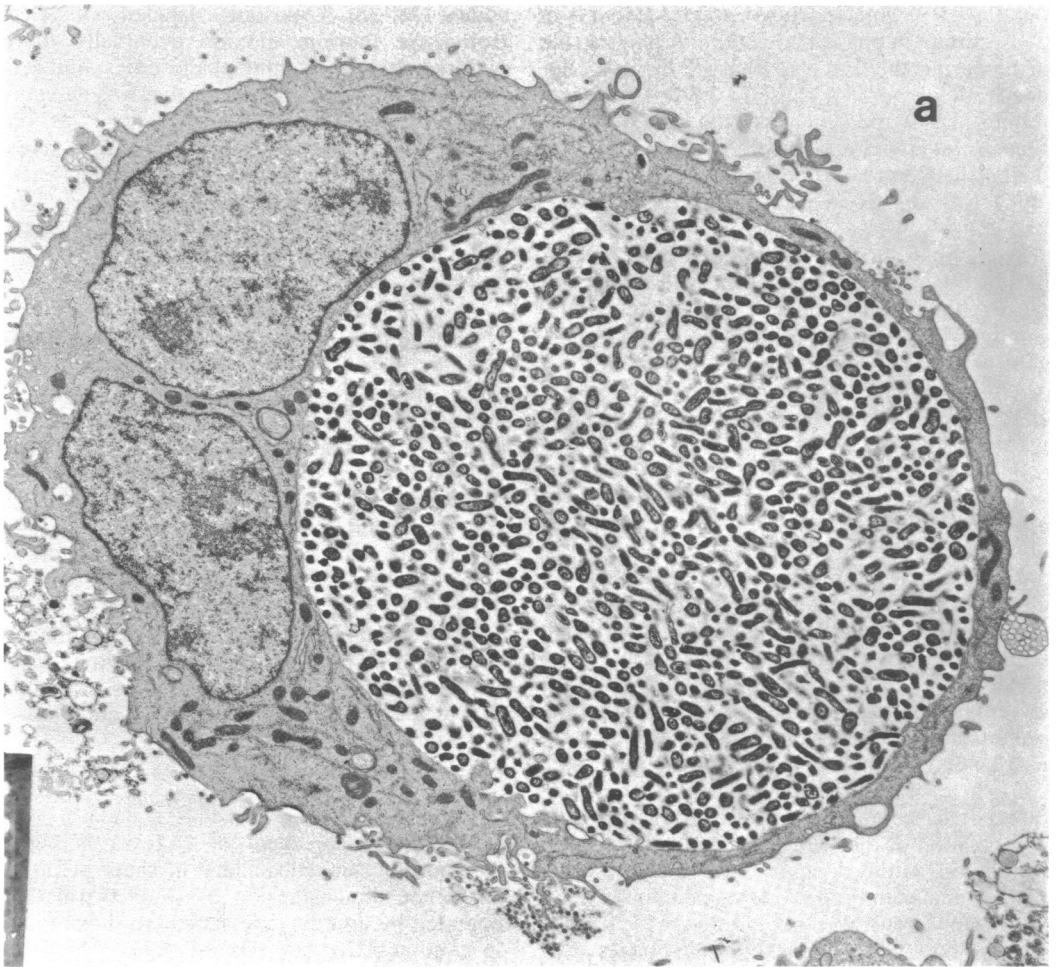

FIG. 1. L-929 cells in suspension culture (a) continuously infected with *C. burnetii* phase I, showing cytoplasmic intravacuolar proliferation, with L cell nuclei pushed to the edge of the cell (38). J774 cells in monolayer culture (b), 27 days p.i. with *C. burnetii* phase I, showing cytoplasmic intravacuolar proliferation. Marker, 1.18 μm (13).

structural and chemical analyses clearly indicate that the agent is morphologically more similar to gram-negative than to gram-positive bacteria.

Several workers have examined ruthenium red-stained phase I and phase II cells by electron microscopy. Burton et al. (37) detected a "fuzzy" layer approximately 20 nm thick on the surface of both phases, whereas Ciampor et al. (43) found it only in phase I cells. The nature of the host may influence the apearance of such a layer, since in one case (37) the rickettsiae were propagated in L cells and in the other (43) they were propagated in yolk sacs.

Genome

Recently the genome size of *C. burnetii* was determined by the method of DNA renaturation to be approximately 1.04×10^9 daltons (138). This is comparable to the genome size of several other rickettsial species and is well within the range of several free-living bacteria, including *Mycoplasma* spp. (5×10^8 daltons). Theoretically, there appears to be sufficient coding information to allow axenic growth of the parasite; its obligate nature is probably due to a few subtle genomic lesions.

The DNA base composition reported by several laboratories (175, 185) is distinctly different from that of other rickettsiae (196). Although we continue to refer to *C. burnetii* as a rickettsia, it is apparent that it differs from the true rickettsiae and calling it "rickettsia" is now due to custom and convenience. We may speculate that *C. burnetii* and the "true" rickettsiae, both of

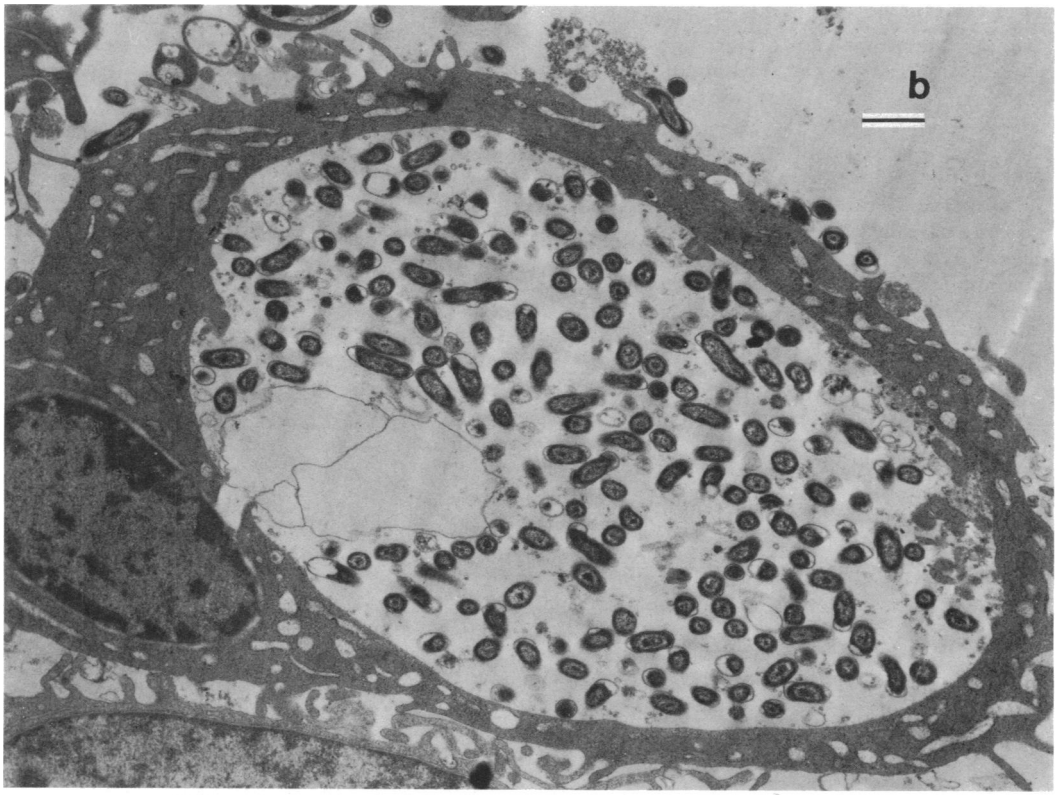

FIG. 1.—*Continued*

them obligate intracellular procaryote parasites, may actually represent an example of convergent evolution.

For some years rickettsiologists have been searching for the existence of a plasmid in *C. burnetii*. Such a plasmid, consisting of 36 kilobases, has now been discovered by L. Mallavia's group in phase I *C. burnetii* and is presently being fully characterized (personal communication).

Ribosomes

The first direct proof of the existence of ribosomes in any of the rickettsial species was reported in 1973 with the isolation of 70S particles from *C. burnetii* that dissociated into 50S and 30S subunits (15). This indicated that *C. burnetii* had conventional procaryotic protein synthesizing machinery, as suggested by earlier reports that cell-free preparations of the agent could incorporate amino acids into a trichloroacetic acid-insoluble fraction (125). This was preceded by the extraction from whole rickettsial cells of RNA species comparable in size to those found in procaryotic ribosomes, 16S, 23S, and 4 to 5S (192). More recently, immunological studies have shown that *Escherichia coli* ribosomal structural proteins L7/L2 and elongation factor EF-G have their counterparts in *C. burnetii* (12). Furthermore, *C. burnetii* ribosomes and elongation factors are capable of functioning, in vitro, with complementary components from *E. coli* (see "Biochemistry of *C. burnetii*," below). Two-dimensional gel electrophoresis of *Coxiella* ribosomal proteins have revealed an array of proteins with a distinctly different pattern than those of *E. coli* (12). These combined studies point out that this parasite's translation apparatus is unremarkably similar to that of other well-studied procaryotes.

Electron microscopic examination of thin sections of *C. burnetii* reveals a dense central nucleoid region composed of fibers 2 nm in diameter (37, 114, 153, 189). The dense core, surrounded by a clear zone, is encompassed by a granular area containing ribosomes (37).

Replication

Although the agent has never been directly observed to divide, ultrastructural studies provide ample evidence of binary fission: constriction of the equatorial region with the concomitant appearance of two nucleoid regions (4, 37,

143, 153, 186). Occasionally the organism appears in chains, which is additional evidence of binary fission. Reports unsubstantiated in other laboratories have suggested the presence of a virus-like stage in the life cycle of *C. burnetii* (113, 114, 171). This claim has been made on the basis of filtration and infection studies. Until substantiated by others with concrete evidence, these reports must remain open to question.

Recently another, as yet unsubstantiated report has suggested the genesis of endospores in a "life cycle" of *C. burnetii* (132). This ultrastructural electron microscopic report bases its conclusion solely on the appearance of an electron-dense body at the pole of the rickettsiae. This attractive possibility, which would neatly correlate *C. burnetii*'s ability to resist various physical-chemical treatments and the above-mentioned "viral" stage, awaits substantive proof and confirmation in other laboratories, including the demonstration of viability, isolation and germination, and putative thermodurability, and possible presence of marker biochemicals such as dipicolinic acid.

Several reports present electron microscopic evidence suggesting that *C. burnetii* exhibits two morphological forms, a large and small form, distinct from the phase variation phenomenon (see below) (87, 132, 199, 208). When centrifuged to equilibrium in cesium chloride or in density gradients prepared with sucrose, Renografin, or Ficoll, the cells separated into two distinct bands, one containing a rod-shaped cell of low density and a second band containing larger, round or coccobacillary-shaped cells that banded at higher density. Two hypotheses have been advanced to explain this: the large cells result from degeneration of the small cells (199, 208), or both cell types represent forms of a complex life cycle (132, 208). McCaul and Williams (132) further speculated that the "large" form is the progenitor of the putative "spore" mentioned above. The so-called spore then purportedly germinates, forming the hardy smaller cell which in turn differentiates into the larger less resistant cell form.

Phase Variation

Based on immunological tests, *C. burnetii* exhibits a phenomenon designated as "phase variation" (30, 65, 188). The phases were originally distinguished serologically. Injection of live phase I or II *C. burnetii* into guinea pigs resulted in antibodies to both phases; phase II agglutinins and complement-fixing antibodies appeared first, later followed by phase I antibody formation (24, 66). In nature or in laboratory animals the parasite exists in the "phase I" state; repeated passage of phase I organisms through embryonated eggs results in conversion to the "phase II" state. Injection of phase II *C. burnetii* into guinea pigs, mice, and hamsters results in reversion to phase I (100). This phase variation is apparently due to surface antigenic differences (66); the identity of the antigens are now being identified and include, apparently, a toxic LPS (see below). Most certainly there are additional phase-specific antigens (198). Phase variation has been observed in infected cell cultures of L-929 cells and the macrophage-like cell line P388D1 (13, 38; Stueckemann, Ph.D. thesis). In both cell types, phase I *C. burneti* converted to phase II (but not in toto) during prolonged infection of the cell lines (several months to over a year). Cloned phase I and II rickettsiae were used in the experiments with the P388D1 cells (13). Within these latter cells, phase II *C. burnetii* remained in phase II.

The fundamental mechanism(s) for phase variation remains unknown. It may be due to inherent genetic differences between phase I and II *C. burnetii* (i.e., two genetically distinct populations of cells, one of which becomes dominant within a host cell). Another possibility is that the organism's gene expression is influenced by the host's environment; i.e., factors inherent to the host cell may suppress or induce expression of the organism's phase characteristics. A third explanation for the variation may be due simply to host lysosomal enzymatic modification of the rickettsial surface, resulting in exposure or generation or both of new antigenic determinants. The L-929 and macrophage cell lines may prove valuable in probing the basis of phase variations.

Morphologically the two phases are indistinguishable, but they do exhibit several biological and chemical differences. Equilibrium density gradient sedimentation in cesium chloride differentiates phase I ($d = 1.33$ g/cm^3) and phase II ($d = 1.22$ g/cm^3) organisms (87). Phase I cells are more virulent for experimental animals than are phase II cells (99). Cytochemical techniques coupled with electron microscopy indicate surface charge differences: phase II rickettsiae display surface anionic binding sites which are absent from the phase I organisms. The carbohydrate compositions and carbohydrate and protein concentrations differ between phase I and II envelopes (92). Both envelopes possess glucose and galactose, but only phase I envelopes contain glucuronic acid. Both phases possess a gram-negative-type LPS, but there are distinct quantitative and qualitative differences with respect to sugar composition and total LPS content (see below). Other differences between the two phases include spontaneous clumping of the phase II rickettsiae (68) and differential uptake by phagocytes; phase II *C. burnetii* are taken up more readily (102, 112; Baca, unpublished data).

LPS

Endotoxic LPS characteristic of gram-negative bacteria was first demonstrated and partially characterized in phase I *C. burnetii* by Baca and Paretsky (17, 18) and Chan et al. (41) and subsequently by Schramek and Brezina (176). The LPS of phase II *C. burnetii* was later characterized (16, 177). Other workers had probably extracted endotoxigenic material, but had not chemically or physiologically characterized the substance as endotoxin. Phase II *C. burnetii* contains one-tenth the amount of extractable LPS found in phase I cells (16). Chemical characterization of the LPS of both phases revealed the presence of a variety of sugars and fatty acids, including those characteristic of gram-negative bacterial endotoxic LPS: β-hydroxymyristic acid and heptoses (17, 41, 176). The presence of glucosamine and fatty acids, including β-hydroxymyristic acid, indicated a lipid A moiety, the toxic entity of endotoxin. Antilipid A antibodies were elicited in rabbits, using mildly hydrolyzed LPS from *C. burnetii* (178). Baca et al. (16) compared the chemical composition of the LPS's isolated from phases I and II *C. burnetii*. Phase II LPS was extracted from cloned as well as noncloned phase II cells which had been serially passaged 95 times through embryonated eggs. Complement fixation and immunodiffusion tests indicated immunological identity of both LPS species (152). Characteristic of other bacterial LPS, phase I and II LPS caused gelation of *Limulus* lysates; phase I LPS induced DNA synthesis in guinea pig leukocytes, nonspecific resistance in mice to virulent *Candida albicans*, and the dermal Schwartzman reaction (152, 176). Thirteen of the 14 sugars found in phase I LPS were also present in phase II LPS, although some of the sugars were present in different proportions (i.e., glucose comprised 20% of the total phase I neutral sugars, but only 2 to 3% of the phase II neutral sugars). The fatty acid profiles of phase I and II LPS were identical. In contrast, Schramek and Mayer (179) reported that phase II LPS had only two of the nine sugars found in phase I LPS. The differences between the two reports (16, 179) may be attributed to the passage history of the rickettsiae. Schramek and Mayer (179) had isolated the LPS from phase II *C. burnetii* which had been serially passaged at least 163 times in their laboratory and an uncertain number of times elsewhere. The compositional differences between phase I and II LPS may reflect the degree of transition of *C. burnetii* to phase II.

Phase I LPS induced pathophysiological changes in experimental animals similar to those associated with endotoxins of gram-negative bacteria (18, 176). Some of these effects in guinea pigs were liver enlargement, hyperthermia, leukocytosis, increased levels of hepatic and serum cortisol, increased incorporation of precursors into hepatic rRNA and plasma protein, loss of body weight, chicken embryo lethality, and hypothermia in rats. Another physiological effect is that phase I LPS extract, in combination with a mycobacterial glycolipid, induced tumor regression in experimental animals (104). Mice injected intraperitoneally with purified and killed *C. burnetii* were then protected from ascites tumor development resulting from injection of sarcoma 180 cells (101); there was the possibility that the protective effect was mediated by the LPS present in the killed *C. burnetii*.

Tzianabos et al. (197) recently examined the cellular fatty acid composition of several rickettsial species and concluded that the fatty acid profile of *C. burnetii* was clearly different from that of the other rickettsiae examined (*R. rickettsii*, *R. typhi*, *R. canada*, and *Rochalimaea quintana*). Unlike the other rickettsiae, which contained straight-chain saturated and unsaturated fatty acids, *C. burnetii* possessed large amounts of iso and anteiso branched-chain fatty acids. Interestingly, the *C. burnetii* fatty acid profile was strikingly similar to that of several *Legionella* species, but DNA hybridization studies showed no relatedness between these two organisms.

BIOCHEMISTRY OF *C. BURNETII*

Problems attendant to a fuller knowledge of the biochemistry of *C. burnetii* are largely components of the general problem of the nature of host-obligate parasitic interrelationships. What are the intrinsic enzymes of *C. burnetii*, and what are the host's contributions which permit proliferation of the parasite? *C. burnetii* proliferates within phagolysosomal vacuoles in the animal and in animal cells in culture (14, 36, 83, 105) (Fig. 1). Does the rickettsia use lysozymic products within the vacuole as energy sources or anabolic precursors or both? How is the parasite protected inside this hostile degradative environment? Does the organism's cell envelope have unusual substituents which regulate its permeability?

Glycolytic and Related Enzymes

Early studies had shown that typhus rickettsiae oxidized glutamate, pyruvate, and succinate (28, 47, 203) and coupled glutamate oxidation with ATP synthesis (25). These rickettsiae incorporated [^{35}S]methionine and [^{14}C]glycine from an axenic medium in reactions requiring (26, 27) NAD, ATP, and glutamate; the previously demonstrated oxidative phosphorylation explained the role of glutamate, and the endergonic syn-

thesis of protein accounted for the need of ATP.

Probably the first studies on the biochemistry and physiology of *C. burnetii* were those relating to energetics and carbohydrate metabolism. ATPase and ADPase activities were found in intact cells by Paretsky et al. (157). Reacting such cells with oxalacetate and acetyl-coenzyme A in the presence of NAD and ATP yielded small amounts of citrate; using cell-free preparations from disrupted cells catalyzed a ninefold increased synthesis of citrate (157). The implied role of a membrane barrier to substrate permeability was strengthened when it was shown that intact cells could not reduce NAD in the presence of malate and glutamate, but that cell-free preparations readily reduced NAD (157). Disrupted *C. burnetii* oxidized malate in the presence of NAD. The oxidation was inhibited by *p*-aminobenzoic acid (PABA), but was reversed by exogenous NAD (80), by a proposed adduct formation with PABA (78), but the phenomenon was unexplained. The proposed adduct formed by PABA and NAD (80) provided a mechanism to explain PABA inhibition of typhus rickettsiae on the basis of PABA binding endogenous typhus NAD, to form a PABA-NAD adduct.

Several laboratories had vainly attempted to demonstrate glucose oxidation by intact typhus rickettsiae, using intact organisms and classic manometric and isotope techniques (28, 147, 203). Weiss (203) attributed the apparent deficiency to the organism's lack of permeases or glycolytic enzymes. Intact *C. burnetii* also failed to oxidize glucose or glucose 6-phosphate, and it was presumed that this apparent metabolic inactivity was due to a permeability problem rather than enzyme deficiency. However, disrupted *C. burnetii* preparations converted glucose to glucose 6-phosphate and oxidized the latter to 6-phosphogluconate (45, 156); isocitrate was also oxidized. The data were not readily accepted; much of the skepticism was based on "unphysiological conditions." The glycolytic data were confirmed and extended in L. P. Mallavia's laboratory where it was shown that cell-free preparations of *C. burnetii* contained glucose 6-phosphate isomerase, fructose 1,6-diphosphatase, aldolase, glyceraldehyde 3-phosphate dehydrogenase enolase, and pyruvate kinase (134a). It was now clear that *C. burnetii* possessed most of the enzymes of glucose dissimilation, and the results implied that the *C. burnetii* cell membrane differed in permeability properties from those of most free-living procaryotes. In addition to the Meyerhof and Entner-Doudoroff glycolytic enzymes, the presence of Krebs cycle enzymes was suggested by low-level oxidations of α-ketoglutarate, succinate, fumarate, malate, oxalacetate, and pyruvate by intact *C. burnetii* (150).

Because *C. burnetii* cell walls contain glucosamine and muramic acid (92), compounds made from glucose-derived precursors, and yet glucose seemingly could not be metabolized by intact rickettsiae, an apparently anomalous situation existed. This question was partially resolved (77). When disrupted *C. burnetii* were reacted with peptidoglycan precursor intermediates, syntheses of acetyl-coenzyme A, UDP-*N*-acetylglucosamine, and UDP-*N*-acetylglucosamine pyruvyl ether were demonstrated, but not those of glucosamine or *N*-acetylglucosamine synthesis (77). The workers suggested that if *C. burnetii* lacked the latter synthases, then the rickettsiae would depend on the host for such intermediates, explaining one factor of obligate intracellular parasitism.

Anabolic Enzymes of Amino Acids, Proteins, and Nucleic Acids

The presence of a broad range of intrinsic rickettsial glycolytic enzymes (134) suggested existence of additional anabolic enzymes. The presence of folic acid in *C. burnetii* (139) implied an active metabolic role for this cofactor. After disrupted *C. burnetii* was incubated with [^{14}C]glycine, ATP, formaldehyde, and exogenous tetrahydrofolate, [^{14}C]serine was isolated (141). The folic acids of *C. burnetii* were isolated and separated by DEAE-cellulose chromatography (131). Although several forms of folates were similar to those of embryonated eggs, a folate unique to *C. burnetii* was found. No final characterization could be made, but the coenzyme had properties of a polyglutamate or prefolic A form. It could not be determined whether the folates were made by the organisms, or whether they were of host origin and modified by the rickettsiae; the general resistance of rickettsiae to sulfonamides suggested the latter alternative. Additional possible participation for rickettsial folates was found in the synthesis of citrulline from ornithine and carbamoyl phosphate and of the pyrimidine precursors ureidosuccinate and orotate from aspartate and carbamoyl phosphate by disrupted *C. burnetii* (124). The presence of autonomous rickettsial nucleic acid biosynthesis was substantiated by subsequent demonstration of RNA polymerase activity in *C. burnetii* (95). The polymerase was DNA dependent, required the four ribonucleoside (AGUC) triphosphates and exogenous energy sources, and was inhibited by actinomycin D and DNase. It was later shown that disrupted *C. burnetii* could synthesize the needed ribonucleoside mono- and disphosphates in the presence of ATP or GTP (42). *C. burnetii* had sufficient endogenous ATP (2 nmol per mg of protein) for its in vitro nucleoside triphosphate synthesis, which would serve as substrates for coordinated

RNA synthesis. The workers proposed that during infection the rickettsiae obtained the necessary nucleoside phosphates from the lysosomal vacuoles of the host cell in which the parasite proliferates. Pyrimidine nucleotide biosynthesis had also been demonstrated by Williams and Peterson (211) in typhus rickettsiae.

Bovarnick's group had reported incorporation of methionine and glycine into protein by intact typhus rickettsiae (26, 27); the obligate intracellular parasitic rickettsiae clearly had endogenous cellular machinery for protein synthesis. Additional evidence for autonomous protein synthesis by rickettsiae was provided by demonstrating incorporation of amino acids into *C. burnetii* protein (125). The proposal that the rickettsiae had endogenous protein-synthesizing machinery was strengthened by the direct demonstration of rRNA in *C. burnetii*; this was the first clear evidence of this critical RNA class in any of the rickettsiae (192). This work was followed by isolation and characterization of *C. burnetii* ribosomes (15) and was likewise the first direct and unequivocal demonstration of rickettsial ribosomes. The ribosomes had typical chemical and physical procaryotic ribosomal structure. Baca (12) later showed that isolated *C. burnetii* ribosomes in the presence of the postribosomal supernatant (S-100) catalyzed translation of polyuridylic acid to polyphenylalanine. The rickettsial translation system had proteins which were antigenically similar to *E. coli* elongation factor G and to *E. coli* ribosomal proteins L7 and L12. Autonomous protein synthesis by *C. burnetii* was more closely investigated by Donahue and Thompson (57–59), who compared translation of polyuridylic acid and Qβ phage RNA by rickettsial and *E. coli* cell extracts. *C. burnetii* had a greater optimum Mg^{2+} requirement (17 versus 6 mM) for polyuridylic acid translation of Qβ phage RNA translation. The kinetics of QB RNA translation were the same for both bacterial systems, with a transit time of 3 to 4 min for the coat cistron, and the phage coat polypeptide product was also the same. The rickettsial extract had no demonstrable mRNA, but the workers proposed that the rickettsiae had the required complement of aminoacyl-tRNA synthetases, tRNAs, ribosomes, and ribosomal factors needed for initiation, elongation, and termination of translation. Donahue and Thompson (58, 59) proposed that the lower number of ribosomes per rickettsia (compared with *E. coli*) could be one of the factors responsible for the rickettsial generation time.

Acidophilic Biochemistry and Intravacuolar Existence

The metabolic evidence cited above indicated that, although viable *C. burnetii* was apparently impermeable to a wide variety of metabolites, the parasite nevertheless maintained itself and proliferated within its host phagolysosomal vacuoles. This perplexing paradox was resolved by Hackstadt and Williams (81, 82), who showed that glucose and certain key intermediates of glycolysis were actively metabolized by intact *C. burnetii* at pH 4.5, but were relatively metabolically inert at pH 7.0!

Analogous to oxidative phosphorylation with glutamate by typhus rickettsiae (25), *C. burnetii* oxidized glutamate at pH 4.5, but not at 7.0, under axenic conditions, to generate an intracellular ATP pool stable for as long as 96 h. The adenylate charge increased during glutamate oxidation with parallel increase in adenylate pool size. Rickettsial viability was directly correlated with the ATP pool, which in turn was more stable at pH 7.0 than at 4.5. Hackstadt and Williams (81, 82) proposed that rickettsial ATP pool stability at pH 7.0, the host's cytoplasmic pH, enabled the rickettsiae to survive until they entered the host's phagolysosomal vacuole. Here the acidic pH would permit rickettsial metabolic activity to begin, thus providing a "biochemical stratagem for obligate parasitism . . . by *C. burnetii*" (81). The observations reconciled previous reports of extremely low levels of oxidation of these metabolites by intact cells at "physiological" pH (150) and of glycolysis by disrupted but not by intact cells (45, 134, 156). The accommodation of *C. burnetii* to an active existence in the phagolysosomal vacuole with a pH of 4.5 to 4.8 could now be explained, substantiating earlier suggestions that *C. burnetii* utilized lysozymal products in its existence (77).

More recently an approach was made to the question of intraphagolysosomal survival by the finding that *C. burnetii* produced superoxide anion and had both superoxide dismutase and catalase activities (2). In light of the Hackstadt and Williams reports (81, 82) it was of interest to note that superoxide was produced at pH 4.5 (the phagolysosomal environment) but not at pH 7.4. On the other hand, catalase activity was greater at pH 7.0 than at pH 4.5. The rickettsial dismutase differed from its host L929 cells (2). Indeed the dismutase-catalase system may afford protection to the parasite not only from its own toxic oxygenated metabolites but also from those generated by the host cell. Myers et al. (142) reported that *R. prowazeki*, which proliferated intracytoplasmically, lacks catalase activity and also fails to produce hydrogen peroxide. Unlike *C. burnetii*, the typhus agent probably does not require catalase for survival because of the lack of hydrogen peroxide in its intracytoplasmic environment (142). The host-independent biochemistry of *C. burnetii* is shown in Fig. 2.

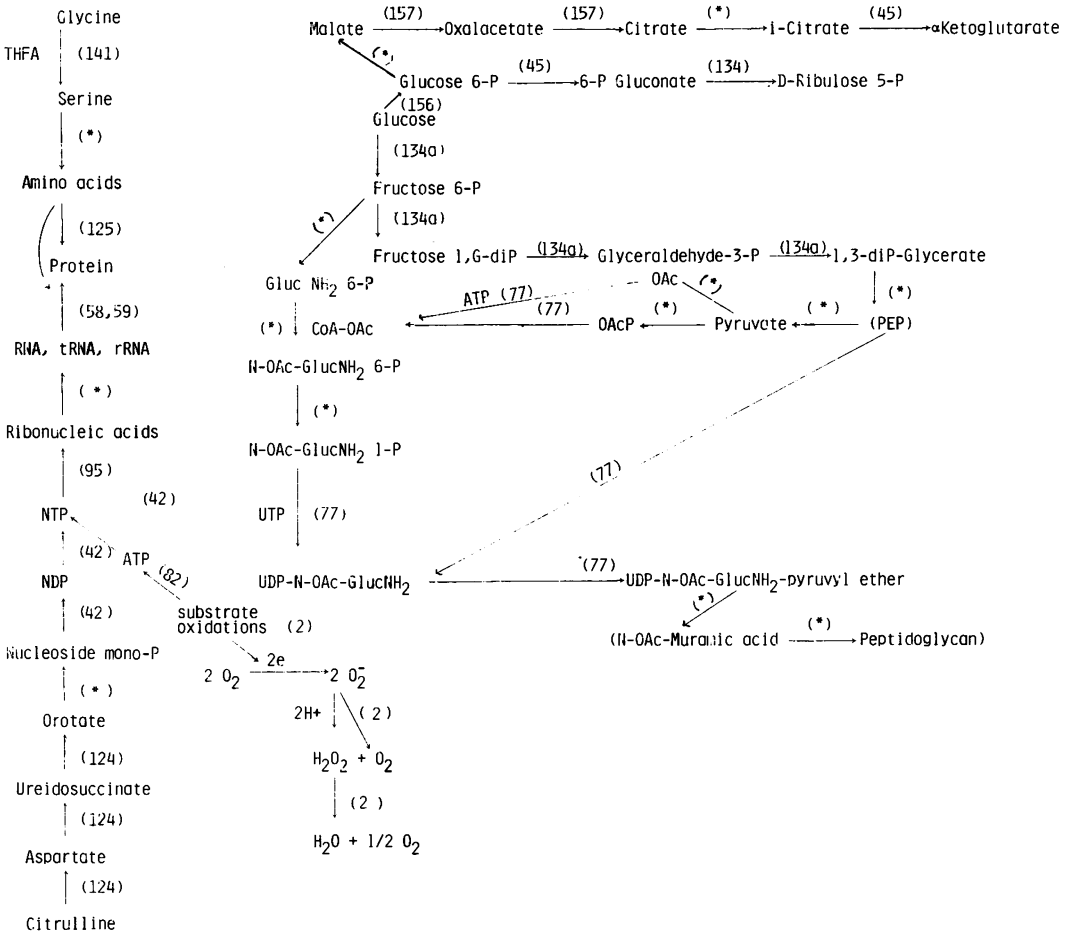

FIG. 2. Host-independent biochemical activities of *C. burnetii*. Reactions as described in Literature Cited. *, Reactions not as yet demonstrated. THFA, Tetrahydrofolic acid.

PATHOBIOLOGY

The biology of *C. burnetii* and the pathobiology of Q fever have been studied less intensively than other aspects of the disease. Investigators have devoted more attention to the ecology, immunology, and epidemiology of the infection because of its public health and economic significance.

The original observations of Derrick (54) and of Burnet and Freeman (35) on the gross pathology of the Q fever described splenomegaly and lymphocytosis in humans and hepatomegaly and splenomegaly in experimentally infected rats and mice.

Humans

Endocarditis can occur as a sequela of Q fever in humans, frequently in latent or chronic cases, often with attendant hepatomegaly and splenomegaly, and occasionally with cardiomegaly, glomerulonephritis, myocarditis, pericarditis, and cardiovascular lesions (39, 46, 64, 67, 75, 107, 127, 169, 172). The rickettsiae themselves have been isolated from the aortic valve (6, 107, 169) as well as from spleen and lungs (6, 169), with cases reported from such diverse locales as England, the United States, South Africa, France, and Iran. The mitral valve was involved as often as the aortic stenoses in endocarditis resulting from Q fever (194). Diagnosticians are again urged to consider Q fever involvement in endocarditis cases where bacterial isolations in usual media are negative. It is said that where Q fever is endemic among farmers, abattoir workers, dairymen and others who work with livestock, Q fever should be considered in the diagnosis of pericarditis (39). The typical Q fever-associated endocarditis case has been described (7) as a middle-aged male, with known

valvular heart disease (usually aortic) and with a history of an influenza-like infection 6 to 16 months previously. Latent Q fever in humans has been shown by the recovery of *C. burnetii* from placentas of women who had experienced Q fever 3 years earlier and who had recovered and were apparently normal (191).

An analogous situation was found in guinea pigs, where latent Q fever was reactivated by X-irradiation or cortisone (180, 181), demonstrating that the disease may persist after its overt clinical features have subsided and that latent endemic foci can recrudesce if the carrier population is appropriately stressed.

Embryonated Eggs

Early work on the effects of Q fever at the cellular and subcellular levels used infected chicken embryos. On the assumption that infection with an obligate parasite led to utilization of host factors by the agent, vitamin concentrations were compared between normal and infected embryonated 16-day-old chicken embryos and yolk sacs (139). Infected yolk sacs had lesser amounts of folic acid (59%), riboflavin (61%), pyridoxine (67%), biotin (73%), and thiamin (85%) material; niacin (93%) and pantothenic acid (103%) levels were about similar and B_{12} was 30% greater in infected material. Infected embryos had lesser amounts of biotin (40%) and pyridoxine (65%); riboflavin (95%), thiamin (118%), and niacin (138%) had similar or greater levels than uninfected embryos. Oxidative phosphorylation of infected embryonic livers was less active than in uninfected embryos. Oxidative phosphorylation of glutamate and citrate by embryonic livers was inhibited by infection. Liver protein synthesis during chick embryogenesis was measured in infected and uninfected embryos of similar physiological age (125). Greater protein synthesis occurred in early (8-day) infected embryos, whereas uninfected embryos had greater synthesis after 10 days. When embryonic age was the parameter, infected embryos had greater synthesis to day 14. It should be noted that the infected embryos' physiological development diminished progressively after 10 days of embryogenesis. The work was extended by chromatographic fractionation of the folic acids of uninfected and infected yolk and yolk sac embryos (131). The folate elution profiles of uninfected and infected tissues were qualitatively identical but infected tissues contained quantitatively less folate, the differences becoming larger during embryogenesis.

The pleomorphic nature of *C. burnetii* was recognized by early workers (52): large and small forms were also found in the cytoplasm of infected yolk sac cells, as were rarely occurring "atypical" forms. They failed to react with *C. burnetii* antibody (4) and might have been the "intermediate" form reported by Rosenberg and Kordova (171). Because of their rare occurrence, it is also possible that the forms were contaminants. In other studies of infected chicken embryos (121), *C. burnetii* was found in all organs of the embryo including the liver, heart, and muscles by 11 days postinfection (p.i.). In more detailed experiments, the infection patterns in chicken embryos of the virulent Tabanidae-Kazakhstan and mildly virulent *Apodemus microtii-Lauga* strains of *C. burnetii* were compared (106). Both strains infected the yolk sac endodermal epithelial cells, and at 13 days p.i. the Tabanidae-Kazakhstan strain had infected 100% of endodermal cells whereas the *A. microtii-Lauga* strain infected 60%. The rickettsiae proliferated within phagolysosomes and in the cytoplasm, and infected cells had lost their glycogen granules. The presence of *C. burnetii* within the cytoplasm was presumed to be due to vacuolar burst. The typical large and small forms of the rickettsiae were observed, as well as very small 30-nm forms. The latter could be inferred to have been the atypical forms described by Anacker et al. (4).

Six-day-old embryonated eggs infected with *C. burnetii* were assayed for glycolytic enzymes over a 10-day period p.i. (135). Until 6 days p.i. there were few differences between the enzymes of normal and infected embryos, but by 10 days p.i. (16-day-old embryos) there were increased specific activities of aldolase (7-fold), phosphofructokinase (7-fold), fructose 1,6-diphosphatase (7-fold), glucose isomerase (30-fold), glyceraldehyde 3-phosphate dehydrogenase (12-fold), enolase (4-fold), and pyruvate kinase (10-fold). Increased enzyme activities were attributed to infection-stimulated hormonal (notably cortisol) activity (135) analogous to the responses of guinea pigs to infection by *C. burnetii* and by its LPS (18, 153, 154, 193).

Guinea Pigs and Other Animals

The ready susceptibility of guinea pigs to infection by *C. burnetii* has made the animal a useful model for studying the pathobiology of Q fever. Guinea pigs infected with a low-virulence strain of *C. burnetii* (Grita M-44) developed mild endocarditis, hepatitis, and granulomatic and necrotic livers (93, 94). Animals infected with the more virulent Nine Mile strain had elevated blood glucose and greater activities in serum of alkaline phosphatase, glutamic oxalacetic transaminase, and β-hydroxybutyric dehydrogenase 3 to 5 days p.i.; creatine phosphokinase activity increased both 3 and 10 days p.i. At 10 days p.i. hypoglycemia, hypophosphemia, splenomegaly, and hepatomegaly developed. Epicarditis with

myocardial lesions became visible at 6 days p.i. Experimentally induced respiratory infection in guinea pigs resulted in interstitial pneumonia accompanied by lesions of lungs, spleen, liver, and heart, lymphoreticular myocarditis, and splenomegaly (165). Plasma copper, seromucoids, and lysozyme concentrations increased three- to fourfold 8 to 10 days p.i., coincident with pyrexia. Plasma zinc as albumin-bound zinc decreased, probably reflecting a redistribution of tissue zinc rather than an actual loss from organs. Plasma copper in the form of ceruloplasmin reflected changes in this protein, and the seromucoid increases were correlated directly with the serum globulin levels. It was proposed that changes in proteins and trace metals resulted from factors released from macrophages which had interacted with the rickettsiae.

A series of studies identified the nature of some aspects of the pathobiology of Q fever in guinea pigs. Intraperitoneal infection of guinea pigs with *C. burnetii* produced pyrexia within 24 h, with pronounced splenomegaly and hepatomegaly by day 3 p.i. (158). Hepatomegaly was maximal at 84 to 96 h p.i., due chiefly to fatty infiltration (158). Liver lipids increased by more than 300%. The lipid classes were predominantly triglycerides, with lesser amounts of cholesterol and unesterified fatty acids (21), but with unchanged concentrations of phospholipids; plasma phospholipids increased. Lipase activity increased simultaneously in the adipose depots, suggesting fat mobilization from the depots into the plasma and liver. Hepatomegaly due largely to steatogenesis is a regular feature of Q fever. Among the causes for fatty liver development could be enhanced synthesis of lipids within the liver or transport of lipids into the liver with failure to export the lipids, leading to lipid accumulation. Impaired export in turn could result from defective membrane structure or deficient chylomicrons. Since infected livers showed no increased lipid synthesis (T. Haney and D. Paretsky, unpublished data), the liver plasma membrane peptides were examined. Although the polypeptide profiles of uninfected and infected membranes were qualitatively similar, quantitative changes in several peptide species were found (126). Plasma membranes of infected liver incorporated about 50% as much glucosamine as uninfected membranes, inferring lesser glycoprotein synthesis. Involvement of membrane changes in liver pathobiology was not shown, but did raise the possibility of defective permeability. Liver glycogen diminished 24 h p.i. and disappeared 48 h p.i. (157); simultaneously, glycogen synthetase activity decreased to 50% 84 h p.i. [Glycogen synthetase catalyzes the transglucosylation reaction: UDP-glucose + $(glycogen)_n$ = UDP + $(glycogen)_{n+1}$, leading to glycogen synthesis.] The synthesis of UDP-glucose from its precursors (ATP + UDP = UTP; UTP + glucose-1-PO_4 = UDP-glucose) was uninhibited during Q fever (190). This inhibition of transglucosylation may have been one of the first biochemical lesions demonstrated in Q fever pathobiology. The largest loss of glycogen synthetase was in the rough endoplasmic reticulum of the liver, accompanied by a shift from the I form (glucose 6-PO_4 independent) to the D form (glucose 6-PO_4 dependent) (153, 190, 195).

Additional pathobiochemical sequelae in the guinea pig were stimulated RNA and protein syntheses, using [^{3}H]orotate and ^{14}C-labeled amino acid incorporations as indices (190). Stimulated protein and RNA syntheses increased progressively during 96 h p.i., coincident with increased hepatic cortisol concentrations (193). Enhanced synthesis of 28S, 18S, and 4S RNA species was parallel to increased numbers of hepatic ribosomes (193), resembling sequelae in regenerating liver (118, 119) and in cortisone-stimulated DNA-dependent RNA polymerase activity of rat liver (214). Cyclic AMP increased more than 50% in infected liver (126). Further parallels between infected guinea pig liver and regenerating liver were found in the increased concentrations of polyamines during infection (160). (Polyamine synthesis also accompanies nucleic acid synthesis in regenerating liver [118]; this is but one example of a widespread direct correlation of increased polyamine levels in rapidly growing cells and tissues [90, 160].) It was now shown that polyamine concentrations increased in infected guinea pig liver, together with increased activity of the liver RNA polymerases (160), and that as infection progressed there was a shift in the relative proportions of polymerases from class II to class I, the polymerase which synthesizes rRNA. Ornithine decarboxylase and *S*-adenosylmethionine decarboxylase, enzymes which participate in polyamine synthesis, had increased activities during Q fever, and ornithine decarboxylase decreased as polyamines increased, again similar to the events in intoxicated and regenerating livers (90, 118). It was proposed (154, 159, 160) that polyamines were among the regulatory factors of liver RNA synthesis in Q fever, perhaps by catalyzing nuclear protein phosphorylation (9, 89, 116). Paretsky et al. (160) proposed that cortisol, which increased during Q fever, activated ornithine decarboxylase, the initial enzyme of polyamine synthesis, and also stimulated class I polymerase as reported in rat liver (214).

Increased phosphorylation of histone and nonhistone chromatin proteins was subsequently observed in nuclei of infected liver (F. Gonzales, M. Halevy, and D. Paretsky, Abst. Annu.

Meet. Am. Soc. Microbiol. 1982, H92, p. 128). These observations together with the demonstration of increased nuclear RNA polymerase activity during Q fever (160) or after treatment with the rickettsial LPS (154, 155) are compatible with the report of increased phosphorylation of nuclear polyadenylic acid polymerase in hepatoma (170). The findings may aid in identifying the mechanisms for stimulated transcription resulting from Q fever.

When LPS isolated from *C. burnetii* (17, 41) was intraperitoneally injected into the guinea pig, the animal responded with hyperthermia, weight loss, hepatomegaly, and steatogenesis, elevated plasma and liver cortisol, increased RNA, increased plasma and liver protein synthesis, and leukocytosis (18, 154). The responses to LPS were characteristic of those resulting in active Q fever in the guinea pig and strongly suggested a direct role of the rickettsial LPS in Q fever pathobiology. The rickettsial LPS incubated with L-929 cells produced qualitatively similarly increased RNA polymerase activities with a similar proportional shift to class I polymerase (155).

Tumors were induced in guinea pigs with hepatocellular carcinoma line 10 cells (104). Formalinized and purified suspensions of *C. burnetii* caused tumor regressions in 32 to 42% of the cases, but were less effective than BCG-antitumorigenic BCG preparations. Enhanced tumor regressions (63%) were obtained with combinations of mycobacterial glycolipids with either purified, killed rickettsiae or *C. burnetii* LPS in oil droplet suspensions. The possible use of the rickettsial LPS as an antitumorigen was suggested.

Hemolymph cells and organs of ticks infected in vivo with *C. burnetii* "filterable particles" became typically vacuolated with cytoplasmic granulations before appearance of the typical rickettsial forms (171). In this connection Leyk and Krauss (120) demonstrated infectivity of *C. burnetii*-infected yolk sac suspensions filtered through 100-nm pores but were unable to show infectivity of such homogenates when filtered through 40-nm pores.

Cell Cultures

Mouse fibroblast L-929 cells in monolayer culture were infected with *C. burnetii*, which then proliferated abundantly within phagolysosomal vacuoles (36) analogous to the intravacuolar localization described for infected guinea pig liver (83). Histochemical demonstration of acid phosphatase and 5'-nucleotidase within the vacuoles indicated stimulated lysozymal response to infection. In further studies or cell culture infections (38), L-929 cells adapted to suspension culture and Vero cells in monolayer culture were persistently infected with *C. burnetii*. (Vero cells purportedly produced little or no interferon.) The rickettsiae characteristically proliferated intravacuolarly. The engorged and distended vacuoles occupied more than one-half to two-thirds of the cell volume, with more than 10^3 organisms per vacuole not uncommon (38). Vero cell vacuoles showed greater histochemical evidence of acid phosphatase activity than did the L cells, and the L cells in suspension culture apparently had lesser activity than did the infected monolayer L cells (38).

Baca et al. (14) observed the fate of phase I and II *C. burnetii* in several macrophage-like murine tumor cells. Phase I rickettsiae established persistent infections in strain P388D1, J774, and PU-5-IR cells, but not in WEHI-3 and WEHI-274 cells; phase II rickettsiae parasitized all five strains, and the rickettsiae proliferated intravacuolarly. Of special interest was the conversion of phase I cells to phase II in P388D1 cells similar to the observations in L-929 cells (Stueckemann, Ph.D. thesis).

The plasma membrane peptides of L-929 cells in suspension culture persistently infected with *C. burnetii* showed greater incorporation of [^{3}H]glucosamine (glycoprotein synthesis?) than uninfected cells (126). As in the case of guinea pig liver membrane proteins, there were no qualitative differences between the peptide profiles of uninfected and infected L cells, whereas several peptide species changed quantitatively. The membranes of infected L cells had 40% greater Na^+,K^+-ATPase activity. The results were consistent with those in infected liver plasma membranes (126).

Phase I and II *C. burnetii* which infected guinea pig macrophages grew within phagolysosomal vacuoles (110, 111). The atypical forms seen by Anacker and others were not present. Kishimoto et al. stated that their data failed to support a *C. burnetii* replication cycle as proposed by other workers (110).

IMMUNOLOGY

The role of humoral and cellular immunity in the control of Q fever has been extensively studied. It appears that, whereas both play a role in eliminating *C. burnetii* from experimental animals, cell-mediated immunity is probably ultimately responsible for eliminating the parasite, with specific antibodies accelerating the process (88).

Probably the first report indicating a role for antibody in the control of Q fever was that of Abinanti and Marmion (1), who concluded that mixtures of antibody and *C. burnetii* organisms were not infectious in experimental animals. Subsequent studies on the efficacy of Formalin-killed phase I and II *C. burnetii* vaccines in

humans and experimental animals indicated that antibodies were involved in the resistance that developed against *C. burnetii* antigens (67, 117, 148).

Various studies subsequently revealed that antibody promoted in vitro uptake of *C. burnetii* by macrophages and polymorphonuclear leukocytes (33, 102, 110–112). Hinrichs and Jerrells (86) have provided convincing evidence that cell-meditated immunity is involved in the control of *C. burnetii* infection. Treatment of normal macrophages with a lymphokine-containing supernatant resulted in growth inhibition of phagocytized *C. burnetii*. The same laboratory presented evidence that normal guinea pig peritoneal macrophages cultured in vitro with immune lymphocytes from *C. burnetii*-infected guinea pigs inhibited the growth of ingested rickettsiae. Kelly (103) reported that killed *C. burnetii* activated, in vivo, guinea pig macrophages to produce an extracellular factor which killed *Listeria monocytogenes*. Phase I LPS also activated the macrophages in vitro. Kishimoto and Burger (108) reported that peritoneal macrophages isolated from guinea pigs exposed to *C. burnetii* exhibited migration inhibition as early as 3 days postexposure and at a time when there was no detectable circulating antibody to phase I antigen. The same group showed that killed phase I *C. burnetii* vaccine induced a cell-mediated immune response in guinea pigs, as detected by lymphocyte transformation and inhibition of macrophage migration (109). Euthymic mice cleared *C. burnetii* from the peripheral circulation and spleen within 14 days after exposure, whereas in athymic (T-cell-deficient) nude mice the rickettsiae were still present 60 days postexposure (109). Both the athymic and euthymic mice produced antibody against the rickettsiae. Humphres and Hinrichs (88) found that antibody indeed altered the course of *C. burnetii* infection in mice by accelerating the initial interaction of the inductive phase of the cellular immune response, which subsequently promoted a more rapid development of the activated macrophages and, ultimately, control of the parasite. Treatment of athymic mice with immune serum 24 h before challenge with *C. burnetii* had no effect on rickettsial multiplication within their spleens.

Several studies have compared the uptake and fate of phase I and II *C. burnetii* by polymorphonuclear leukocytes and cells of the monocytic series. Live or killed phase I rickettsiae were phagocytized to a lesser extent than phase II organisms by normal mouse or guinea pig peritoneal macrophages and polymorphonuclear leukocytes (33, 102). Phagocytosis was enhanced by rabbit immune serum containing antibodies versus phase I; phase II antibodies had no such enhancing effect. A slight increase in phagocytosis of phase II *C. burnetii* was observed with sera containing both antibodies. Similar results were obtained with normal and immune human leukocytes and antibodies, but with no increased uptake of phase II organisms when antisera were included (213). Downs (60) reported that *C. burnetii* multiplied in both immune and nonimmune monocytes, with phase I multiplying to a greater extent. Hinrichs and Jerrels (86) reported that *C. burnetii* was readily phagocytized by normal guinea pig macrophages and that it was not destroyed, but rather proliferated in and destroyed the macrophage. In the latter study, specific antiserum added to the macrophage culture before or after infection, or reacted directly with rickettsiae, failed to prevent intracellular infection. Kishimoto's group (110–112) examined the fate of *C. burnetii* in macrophages and confirmed the observation by Kazar et al. (102) that phase I *C. burnetii* is more resistant to phagocytosis by macrophages from normal and immune animals than are phase II organisms. Pretreatment of phase I and II rickettsiae with normal serum did not affect their ability to multiply and destroy macrophages from either normal or phase II-immunized guinea pigs. In contrast, only phase I organisms were destroyed by macrophages from phage I-immunized animals in the presence of normal serum. Apparently the enhanced in vitro rickettsicidal capacity of macrophages from phase I-immunized animals against homologous organisms was not attributable to enhanced lysosomal hydrolase activity since no significant differences in the specific activities of various lysosomal hydrolases were observed between macrophages obtained from uninfected controls and phase I- or II-immunized animals. Immune serum potentiated the destruction of both rickettsial phases in normal macrophages; these results are opposite to those obtained by Hinrichs and Jerrels (86).

Some of the apparently contradictory results obtained by the different laboratories might be partially reconciled by the use of phagocytes obtained directly from animals. A distinct problem with macrophages derived from various animals is the functional heterogeneity exhibited by such phagocytes (97, 201). Results obtained with such cells must be interpreted with caution. Subsequently, one of us (O.G.B.) recently examined the fate and interaction of both phases on *C. burnetii* in several macrophage-like tumor cell lines of murine origin. These cell lines exhibit population homegeneity and different capacities to interiorize particles and have been extensively characterized with respect to phagocytic capabilities (137, 202). With these cell lines, it was determined that they exhibited different capacities to interiorize *C. burnetii* and

furthermore, although phase II *C. burnetii* infected all the cell lines tested (J774, P388D1, PU-5-IR, WEHI-3, WEHI-274) at a multiplicity of infection of 500, phase I *Coxiella* only infected J774, P388D1, and PU-5-IR (14). Only at multiplicities of infection exceeding 3,000 did phase I *C. burnetii* infect the WEHI-3 cells (Baca, unpublished data). Phase I antibody did accelerate the entry of phase I rickettsiae into the cell lines (14); however, the antibody did not prevent the establishment of a persistent infection (Baca, unpublished data). Although it appears that antibodies play a role in controlling Q fever, they may also promote infection by accelerating entry of the parasite into host cells.

Vaccines

Marmion (128) and Kazar et al. (98) reviewed the status of Q fever vaccines. Experimental vaccines included killed and attenuated *C. burnetii* and extracts of the agent. Unfortunately most, if not all, of these vaccines induced severe local skin reactions. Phase I *C. burnetii* vaccines were the most effective in providing protection to challenge with live phase I rickettsiae. Formalin-killed phase I *C. burnetii* were shown to be 100 to 300 times more effective in guinea pigs in eliciting antibody and protection to challenge with live organisms. An attenuated *C. burnetii* strain designated Grita M-44 and apparently in phase II was prepared by Genig (71) and tested in Soviet volunteers with success. However, recent work with the vaccine in guinea pigs indicated that the organisms persisted in the animals for long periods of time and that the infection could subsequently reactivate (93, 94). These observations sharply bring into question the use of such live vaccines in humans where reactivation of previous infection has been reported (191). Extracts from *C. burnetii* have also been tested in human volunteers. A trichloroacetic acid extract of phase I *C. burnetii* caused a significant rise in phase I antibody titer in persons tested; reactions to the vaccine were more severe in those with a previous history of Q fever (98). Significant progress in solving problems of toxic reactogenicity of *C. burnetii* vaccines was recently reported by Williams and Cantrell (210). They demonstrated that vaccines of killed phase I *C. burnetii* produced immunity but also pathological responses in an endotoxin nonresponder strain of mice. However, when killed phase I rickettsiae were extracted with chloroform-methanol and the cell-free residue or the chloroform-methanol extract was injected into the mice, no pathological responses were induced. Furthermore, the cell-free residue elicited antibody production against phases I and II of *C. burnetii* and also protected mice against subsequent challenge by live rickettsiae. The chloroform-methanol extract fraction failed to induce either antibody or protection. The report proposed that the pathology-producing factors of whole-cell vaccines could be extracted with chloroform-methanol without impairing the immunogenicity of the cell-free residue. This interesting report should contribute to the production of an effective, nontoxic reactogenic vaccine against Q fever. Further progress in this promising study is awaited to clarify the chemistry and biological properties of the chloroform-methanol extract and to provide additional immunological information on the reactions of primates and other experimental animals to the cell-free residue. There are no commercial vaccines presently available for human use in the United States, and until the problems of pathological reactogenicity are fully overcome a vaccine is unlikely to become available for public use.

SUMMARY AND PROSPECTS

"It is better to ask some of the questions than to know all of the answers." (James Thurber, "The Scotty Who Knew Too Much," *in Fables for Our Time*, [Harper & Row, New York, 1940]).

Forty-five years after Derrick and Burnet described Q fever and its rickettsioid etiological agent, the disease remains relatively obscure to the general lay and scientific audiences, lacking the dramatic appeals of the classic rickettsioses. Even for workers with Q fever, aspects of the immunology and pathobiology of the infection, the biology of *C. burnetii*, even the taxonomic position of the organism, and the molecular basis of the parasite-host interrelationships remain inadequately answered questions.

"Obligate intracellular parasitism" by its very phrasing connotes a complete host dependency by an organism. As the parasite's biochemical (enzyme, metabolite), physiological (cell envelope, intracellular organelles), physical and chemical (environmental pH and redox potential) deficiencies become increasingly understood, and when such deficiencies are corrected by appropriate exogenous supplementations, the "parasite" becomes instead "fastidious." When more complete information is obtained, axenic cultivation can be attained. It was said 10 years ago, "One would be led to believe . . . that *C. burnetii* is ideally suited for the study of metabolic reactions and may well serve as the prototype of obligate intracellular bacteria. *Unfortunately, nothing is further from the truth* [italics ours]. Not only do intact resting cells display very little metabolic activity . . . (but) one must conclude that resting cells of *C. burnetii* are relatively inert" (203). A large complement of anabolic and catabolic enzymes have now been identified in *C. burnetii*; many more will be

uncovered. The parasite has been shown to have an acidophilic metabolic permeability, compatible with its phagolysosomal existence. The genome has been extracted and its molecular size (10^9 daltons) is of the same magnitude as those of free-living procaryotes, opening the prospects of genomic analysis with the tools of molecular biology. This will add to our knowledge of the organism's biology. Finding a plasmid in *C. burnetii* now opens experiments for gene transfer in this rickettsia. It is likely that the next decade will witness identification of the nature of *C. burnetii*'s host dependence, followed by axenic cultivation of the organism. Solving the problem of obligate intracellular parasitism of *C. burnetii* will prove fruitful for work with other rickettsiae and other obligate parasites. The biology of *C. burnetii* will be further elucidated with the modern techniques and information of molecular biology and biochemistry, including genome analysis and genetic recombination. Definition of the chemical, physical, and biological properties of the agent's cell envelope will help explain the basis for the intravacuolar habitat and proliferation of *C. burnetii* and, by extrapolation, that of other intracellular parasites.

The general strategies which procaryotic intracellular parasites have evolved to thwart their destruction within macrophages and polymorphonuclear leukocytes include prevention of fusion of the phagocytic vesicle with lysosomes (e.g., *Mycobacterium tuberculosis, Chlamydia psittaci*) and escape from the phagocytic vacuole into the cytoplasm, thereby avoiding lysosomal attack (*R. prowazeki*). Other procaryotic intracellular parasites, including *Salmonella* spp., *Brucella* spp., and *C. burnetii*, actually thrive and proliferate within the normally hostile phagolysosomal milieu. The biochemical-biophysical interactions between parasite and host which result in survival of the parasite are as yet largely unknown. It may be speculated that *C. burnetii* has evolved a hydrophobic cell envelope enabling the parasite to resist attack by lysosomal enzymes. Recent demonstrations that *C. burnetii* is permeable to metabolites at acidic, but not at physiological pH levels suggest that rather than serving as a substrate for lysosomal enzymes, *C. burnetii* can actually utilize the products of lysosomal hydrolysis of cell constituents as precursor substrates for rickettsial metabolism. The host-parasite interactions leading to cellular entry of *C. burnetii* will become better understood as a result of continued experiments with the rickettsia and different host cell lines in culture.

Q-fever-induced phosphorylation-dephosphorylation of liver chromosomal substituents, polyamine synthesis, and hormone activation correlate the stimulated hepatic transcription and translation of regenerating liver. Together with glycogen depletion and steatogenesis in the infected liver, the pathobiological events resulting from Q fever have parallels in several procaryotic and eucaryotic infections, in regenerating liver, hepatomas, tissue hypertrophies, and other pathologies. Q fever will continue to serve as an excellent model system for clarifying the molecular basis for many such phenomena and for the study of general problems of host-parasite interrelationships.

Because of the widespread distribution of its endemic reservoirs and multiple routes of infection, Q fever will likely remain a disease of economic and public health importance in spite of successful chemotherapy and preventive measures such as milk pasteurization. From a medical standpoint, latent infections and sequelae such as endocarditis should attract increased attention as diagnosticians become more aware of the prevalence of Q fever.

There are challenging questions to be answered: how does *C. burnetii* enter the host cell and participate in phagolysosomal vacuole formation? What nutrilites does the parasite obtain in the vacuole? What are the biochemical and biophysical conditions necessary for axenic cultivation of the organism? What are the properties of the rickettsia's cell envelope which contribute on the one hand to its permeability to metabolites at acidic pH values and on the other hand to its resistance to lysosomal enzymes? What are the mechanisms which trigger development of host cell and organ pathologies? What is the mechanism of phase conversion? What role, if any, does the plasmid play in the biology of *C. burnetii*? Can the parasite's genome be cloned so as to permit development of an effective vaccine against Q fever, without producing harmful side effects? The next decade should witness answers to some of these questions by using the information and technology of modern biochemistry and technology combined with the necessary adjunct of ingenious experiments.

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